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PATENT APPLICATION

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Valerie CHEYNET-SAUVION et al.

Application No.: 09/402,131

Filed: December 8, 1999

For: RNA-DEPENDENT RNA POLYMERASE FUNCTIONING PREFERABLY ON RNA MATRIX AND PROMOTER-DEPENDENT TRANSCRIPTION PROCESS WITH SAID RNA-DEPENDENT RNA POLYMERASE

Group Art Unit: 1655

Examiner: B. Sisson

Docket No.: 104458

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D.C. 20231

Sir:

Prior to initial examination of the continued prosecution application filed January 16, 2002, a three-month suspension having been requested, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Page 21, lines 17-31, delete current paragraph and insert therefor:

D1

The introduction of nonsilent mutations, with the aid of PCR techniques, into one or more cassettes of the modular gene previously defined, led to genes encoding polymerases exhibiting an amino acid sequence differing by at least one amino acid with respect to the T7 expressed from the modular gene. Mutant genes were in particular prepared which encode at least one modified amino acid in the B motif of the wild-type enzyme, for example with an alanine (A) in place of an arginine (R) at position 627 and/or an alanine (A) in place of a serine (S) at position 628 and/or an alanine (A) in place of a lysine (K) at position 631 and/or

an alanine (A) in place of an arginine (R) at position 632 and/or an alanine (A) in place of a tyrosine (Y) at position 639.

D1

[Page 30, line 5 to page 31, line 24, delete current paragraph and insert therefor:]

The reactions are performed in 20 μl of a buffer derived from that described by J.F. Milligan, D.R. Groebe, G.W. Witherell, O.C. Uhlenbeck, Nucleic Acids Res. 15, 8783 (1987), namely Tris-HC1 40 mM, pH 8.1, spermidine 1 mM, PEG 8% (g/V), TRITON X-100 (Octoxynol-9) 0.01% (V/V), BSA 5 μg/100 μl, 1 μl (40 u) of porcine RNAguard (Pharmacia Biotech), UTP 12.5 μM, a 32P UTP 0.5 μCi (Amersham, 10 mCi/ml 400 Ci/mmol) 0.4 mM of the three ribonucleoside triphosphates A, G, C, $Mg(OAc)_2$ 6 mM. The template concentration is set at 10^{11} copies of each strand in 20 μl of reaction. The wild-type T7 RNA polymerase is used at 0.5 μM (100 ng/20 μl), the mutated T7 RNA polymerase R627A at 3.65 μM (730 ng/20 μl). Before adding the enzymes, the reactions are denatured for 5 minutes at 65°C in a heating block and then gradually brought to 37°C. The reactions are initiated by the addition of the polymerases, incubated for 1 hour at 37°C and then stopped by the addition of an equal volume of 2× blue formamide (formamide 90%, EDTA 25 mM, xylene cyanol 0.02%, bromophenol blue 0.02%) and denatured for 5 minutes at 95°C. 20 μl of each reaction are deposited on a denaturing gel (20% acrylamide, urea 7 M, 1X TBE), and then after migration, the gel is autoradiographed at -70°C on a Biomax MR film (Kodak). The results (electrophoretic profiles) are presented in Figure 5, and in particular the transcription results obtained with the mutated T7 RNA polymerase R627A (wells 1-3) and the wild-type T7 RNA polymerase (wells 4-6), on the single-stranded RNA templates (wells 1 and 4), double-stranded DNA (wells 2 and 5), and single-stranded DNA (wells 3 and 6). The transcription on single-stranded RNA, detected by detection of a complete transcript of 33 bases, is possible using the mutated T7 RNA polymerase R627A (well 1) and not the wild-type enzyme (well 4) which produces on the other hand many abortive transcripts; see

D2

nevertheless the different results obtained in Example 3 below. The mutated T7 RNA polymerase R627A exhibits a residual transcription activity on double-stranded DNA (well 2), characterized by the presence of a predominant transcript which is smaller in size than the expected transcript, and the presence of a small quantity of abortive products. On single-stranded DNA (well 3), this transcript of abnormal size disappears, whereas the quantity of abortive products increases. By contrast, the wild-type enzyme allows the production of specific transcripts in the presence of DNA templates (wells 5 and 6), this enzyme exhibiting, moreover, a better transcription activity on the double-stranded DNA template (well 5) than on the single-stranded DNA template (well 6); for these two templates, the wild-type enzyme induces the synthesis of numerous abortive transcripts. These results show that the replacement of the arginine 627 by an alanine confers on the mutant enzyme the possibility of synthesizing RNA from an RNA template and induces the loss of capacity to synthesize RNA from a DNA template.

D2

IN THE CLAIMS:

Please cancel claim 42 without prejudice to or disclaimer of the subject matter contained therein.

Please replace claims 35, 43, 44, 48 and 59 as follows:

35. (Amended) A method of amplifying an RNA target sequence, by transcription under the control of a promoter, in an RNA sample comprising said target sequence, said method comprising bringing said sample into contact:

D3

- with a reagent capable of hybridizing with RNA comprising said target sequence,
- in the absence of deoxyribonucleoside triphosphates,
- and with an enzymatic system comprising an RNA-dependent RNA polymerase,

under conditions allowing the hybridization of said reagent with said RNA comprising said

target sequence and under conditions allowing the functioning of said RNA-dependent RNA polymerase;

wherein said reagent contains:

(i) a first nucleotide strand comprising: a) a first nucleotide segment capable of playing the role of sense strand of a promoter for said RNA polymerase and b) downstream of said first segment, a second nucleotide segment comprising a sequence capable of hybridizing with a region of said RNA, and

D3

(ii) in the hybridized state on the first strand, a second nucleotide strand comprising a third nucleotide segment capable of hybridizing with said first segment so as to form with it a functional double-stranded promoter;

wherein said RNA polymerase (1) is from a family of RNA polymerases whose promoters have a consensus sequence from position -17 to position -1 and (2) is capable of transcribing an RNA template, in the presence of said reagent hybridized with said template, in the absence of associated protein factor and in the absence of a ligase activity.

D4

43. (Amended) A method according to claim 35, wherein said RNA polymerase is from a family of RNA polymerases selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

44. (Amended) A method according to claim 35, wherein said RNA polymerase is derived by mutation from an RNA polymerase from a family of RNA polymerases selected from the group consisting of T7, T3 and SP6 RNA polymerases.

D5

48. (Amended) An RNA polymerase, capable of transcribing, under the control of a promoter, a polynucleotide target of interest of a sequence contained in a polynucleotide template, by synthesizing, in the presence of said template and in the absence of associated protein factor and in the absence of a ligase activity, a product of transcription containing an RNA sequence complementary to said sequence, wherein said RNA polymerase (1) is

capable of synthesizing said product of transcription with a better yield when said target sequence of said template consists of RNA than when it consists of DNA and (2) is from a family of RNA polymerases whose promoters have a consensus sequence from position -17 to position -1.

D5

59. (Amended) An RNA polymerase according to claim 58, wherein said other amino acid residue is an alanine, valine, leucine, isoleucine, glycine, threonine or serine residue.

D4

REMARKS

Claims 35-41 and 43-68 are pending. Claim 42 is canceled and claims 35, 43, 44, 48 and 59 and the specification are amended herein. The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. §1.121(b)(1)(iii)) and claim (37 C.F.R. §1.121(c)(1)(ii)).

Claims 48-68 are withdrawn from consideration. However, due to the interrelatedness of the elected and withdrawn claims, it is respectively requested that all of the claims be examined in a single application. In particular, claims 48-60 are directed to RNA polymerases that have been mutated so as to better function in the method of claim 35. In addition, claim 64 is directed to a method for mutating the RNA polymerase to obtain such a mutated polymerase. Finally, claims 65-68 are directed to methods similar to the method of claim 35, which use either RNA polymerase of elected claim 43 or the mutated polymerases of claim 48. M.P.E.P. §803 states that "if the search and examination of the entire application can be made without serious burden, the examiner <u>must</u> examine it on the merits, even though it includes claims to distinct or independent inventions" (emphasis added). It is respectfully submitted that this policy should apply in the present application in order to avoid unnecessary delay and expense to Applicants and duplicative examination by the Patent Office.

The Office Action objects to the Declaration as being defective because non-initialed and/or non-dated alterations have been made to the Declaration citing 37 C.F.R. §1.52(c), and because it was allegedly not executed in accordance with 37 C.F.R. §1.66 or §1.68. A new Declaration was filed on January 16, 2002. Therefore, the objection should be reconsidered and withdrawn.

The specification is objected to based on the alleged failure to provide generic terminology for the trademark TRITON. The trademark TRITON represents a family of surfactants. The specification has been amended herein in order to identify the trademark TRITON X-100 and the generic terminology therefor. Support for TRITON X-100 can be found in the Milligan article (copy attached) at page 8784, line 2 from the bottom, which is cited in the specification on page 30, lines 5-8, and correctly identified on page 5, lines 35-36, of the specification. Therefore, the objection should be reconsidered and withdrawn.

Claims 35-47 are rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement. Applicants respectfully traverse the rejection.

The present invention is based on the discovery of a new use for a known family of RNA polymerases, which were known to have a capacity for transcribing a double-stranded template of DNA, i.e., for synthesizing an RNA sequence complementary to one of the strands of the DNA template. That is, they were known to be DNA-dependent RNA polymerases. It has been discovered that the family of RNA polymerases also has the capacity for transcribing an RNA template, i.e., for synthesizing an RNA sequence complementary to the RNA template. That is, it has been discovered that these known DNA-dependent RNA polymerases are also RNA-dependent RNA polymerases. It has further been discovered that by mutating the RNA polymerases, mutated RNA polymerases can be easily obtained that are capable of synthesizing a transcriptional product from an RNA template with a better yield than from a DNA template.

The family of RNA polymerases that can be used according to the present invention is one that is capable of transcribing under the control of a promoter having a consensus sequence from position -17 to position -1. See the specification, at page 5, lines 18-23. This family includes the RNA polymerases of phages T3, T7 and SP6.

Of the members of this family of RNA polymerases, T7 RNA polymerase is the best characterized. See the specification at page 13, lines 30-33. T7 RNA polymerase is known as a DNA-dependent RNA polymerase. However, it is shown in the present application to also be an RNA-dependent RNA polymerase. Specifically, Example 2 of the present application shows that a mutated T7 RNA polymerase can transcribe an RNA template. Example 3 further shows that the <u>wild-type</u> T7 RNA polymerase can also transcribe an RNA template. Thus, although T7 RNA polymerase is known as a DNA-dependent RNA polymerase, as noted in the Office Action, this polymerase also has an RNA-dependent RNA polymerase activity. Thus, the recitation of it having an RNA-dependent RNA polymerase activity is not a typographical error, as suggested in the Office Action at page 6, second paragraph. Instead, as shown in the present application, polymerases known to transcribe DNA can also be used by the present method to transcribe RNA.

Phage RNA polymerases are very simple enzymes, which are highly homologous to one another and consist of a single subunit. As discussed in the present specification, the simplicity of these RNA polymerases has made it possible to identify and to define, *with the aid of mutagenesis*, regions or residues involved in the polymerase function. One of the mutagenesis strategies consisted of exchanging elements between T7 RNA polymerase and its close relative T3 RNA polymerase, whose amino acid sequences are 82% identical. This strategy has lead to the identification of the polymerase elements involved in the recognition of the promoter. It has been shown, for example, that the substitution of a *single* amino acid in one of T3 or T7 polymerase allows the mutated enzyme to specifically recognize a

promoter of the other enzyme, i.e., the mutated T3 polymerase recognizes a promoter of T7 polymerase, and *vice versa.* In the same manner, reciprocal substitutions in the respective promoter sequences confer on the mutated promoter the capacity to be recognized by the other enzyme. See the specification, at page 14, lines 7-27.

From the above discussion, it can be concluded that the RNA polymerases whose promoters have a consensus sequence between positions -17 and -1 form a recognized class of RNA polymerases having high similarity. It is further shown by the fact that those skilled in the art refer to all of these polymerases as "T7-like RNA polymerases" and also refer to "T7-like bacteriophages." See, e.g., CHAMBERLIN *et al.* (copy submitted with June 5, 2001, Amendment), at page 88, first paragraph, and page 89, Heading II. It also results from the above discussion that those skilled in the art have a good knowledge of the relationship between the structure and the function of the phage RNA polymerases. See, e.g., R. SOUSA, TIBS 21, 186-190, which was submitted with the Information Disclosure Statement filed October 29, 1999. It should be noted that SOUSA, just as CHAMBERLIN, also expressly refers to "T7-like RNA polymerases." See the abstract, last line.

It should also be noted that each of these polymerases can recognize several different promoters in the phage to which it belongs. For example, the T7 phage recognizes 17 promoters. See the specification at page 5, lines 1-5.

All of the above also suggests that these phage RNA polymerases can adapt to various situations and can accept some degree of variation, including mutations.

As further indicated in the specification, structural *and mutagenic* studies have made it possible to correlate the functional elements of the T7 enzyme with the structural elements. In other words, not only the T7 polymerase has a relatively simple structure, but moreover, two functional domains have been identified: a promoter recognition domain and a catalytic domain. See the specification at page 15, lines 1-7. Moreover, much is known about the role

of small regions of the polymerases and even the role of some single amino acids has been studied in detail. See the specification, from page 15, line 8 to page 16, line 18.

Accordingly, at the time this invention was made, those skilled in the art already had a good knowledge of how to practice mutations in phage RNA polymerases and how to study the effects of such mutations. This is confirmed by the discussion appearing from page 14, line 7 to page 15, line 7 of the specification, which clearly shows that at least since 1990, mutagenesis strategies on T7-like RNA polymerases have been routinely performed by those skilled in the art and are widely described in the pertinent literature.

The specification shows that an RNA polymerase having the ability to transcribe an RNA template in the presence of a promoter having a consensus sequence between positions -17 and -1, in the absence of associated protein factor and in the absence of a ligase activity, **can exist.** The specification also discloses examples of transcription with two such RNA polymerases: one is the *existing* T7 RNA polymerase, which was already known although the new function of transcribing RNA, which the present inventors discovered, was not known; and the other is a mutated RNA polymerase (R 627 A) described in the specification at page 21, second full paragraph.

T7 RNA polymerase is commercially available. The method of constructing the mutated polymerase is sufficiently described at pages 20-21 and in Example 1. In addition, the specification includes specific examples, Examples 2 and 3, describing transcription with both enzymes. Accordingly, all the elements necessary for performing the method of claim 35 are precisely disclosed in the specification. *Thus, no experimentation at all is needed to perform this method.* As a result, the situation is clearly not analogous to *Genentech*, as suggested in the Office Action.

In view of the well recognized similarity of the various phage RNA polymerases having a promoter with a consensus sequence from -17 to -1, it is respectfully submitted that

one of ordinary skill in the art would also be able to use other RNA polymerases of the claimed family and to produce and use mutated RNA polymerases and to verify by routine experiments, as is reported in the examples of the present specification, whether the enzymes can transcribe an RNA template.

In addition, the specification clearly enables the production of mutated RNA polymerases according to claim 48, which can be used in the method of claim 35. As taught in the present specification, an RNA polymerase (RNAP) according to claim 48 can be derived by mutation from a T7-like RNAP. See the specification, at the paragraph bridging pages 9-10; at page 16, lines 19-32; and at page 18, lines 8-13. In addition, as discussed above, the prior art shows that those skilled in the art know how to mutate a T7-like RNAP. Also, a specific method is described in a detailed manner in the present specification from page 20, line 1 to page 23, line 34, and still more particularly described in Example 1. More generally, in order to find new mutated RNAPs, the skilled artisan merely has to introduce, by known methods, a mutation in the amino acid sequence of a T7-like RNAP.

Once a mutated RNAP is obtained, it must be tested for transcription of RNA and DNA templates for determining the yield of each transcription, in order to check whether the yield of transcription is higher with RNA templates than with DNA templates. The methods of transcription are generally known, and a specific method is described in a detailed manner in Example 2.

The skilled artisan searching for new mutated RNAPs may proceed by testing random mutations. This may represent a great number of experiments, but these experiments are merely routine. The skilled artisan may also proceed in a reasoned manner by taking into consideration the considerable knowledge about the structure and functions of known T7-like RNAPs. Moreover, the specification provides further specific guidance, such as introducing a mutation in the region 625-652 of T7 RNAP or in a corresponding region of another T7-like

RNAP. See the specification, at page 18, last complete paragraph. Since the T7-like RNAPs have high similarity, the skilled artisan will be in a position to find, by checking the literature or by performing routine experiments, the region of a given T7-like RNAP that corresponds to region 625-652 of T7 RNAP.

Moreover, in the paragraph bridging pages 18-19, the specification teaches introducing mutations at specific positions (627, 628, 631, 632 and 639). Here again, introducing various mutations at these positions and checking the yield of transcription from RNA templates and DNA templates is mere routine. In addition, the specification provides further help by recommending the introduction of mutations by replacing certain amino acid residues (arginine, lysine, serine, tyrosine) of the wild-type T7-like RNAPs with another amino acid residue. The specification further teaches that the replacement amino acid can be in particular chosen among alanine, valine, leucine, isoleucine, glycine, threonine or serine.

Furthermore, several examples of specific mutated T7-like RNAPs are given at page 21, second full paragraph, including R 627 A, S 628 A, K 631 A, R 632 A and Y 639 A. Still further specific mutated RNAPs are disclosed in the paragraph bridging pages 21-22, wherein the region 625-652 has been replaced in whole or in part by a homologous region present in RNAPs of hepatitis C virus or yeast integrase 32. Here again, the situation is far from being comparable to that of *Genentech.*

Moreover, it results from the above that the specification clearly teaches alternative amino acid sequences for the mutated RNAPs, as well as specific positions and regions that can be predictably modified. Thus, one skilled in the art finds in the specification considerable guidance for searching for, testing and obtaining new mutated RNAPs, and he can proceed with a reasonable expectation of success in view of the high similarity of the T7-like RNAPs.

For all of the above reasons, it is respectfully submitted that all of the claims are enabled by the present application. Therefore, the rejection under 35 U.S.C. §112, first paragraph, should be reconsidered and withdrawn.

In view of the above amendments and remarks, it is respectfully submitted that the present application is in condition for allowance. Favorable consideration and prompt allowance are therefore respectfully requested.

Should the Examiner believe that anything further would be required in order to place this application in condition for allowance, the Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below.

Respectfully submitted,

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WPB:MLM/ja

Attachments:
Appendix
Milligan Article

Filed: April 15, 2002

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DEPOSIT ACCOUNT USE
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